Interleukin 6 Knockout Prevents Angiotensin II Hypertension
Role of Renal Vasoconstriction and Janus Kinase 2/Signal Transducer and Activator of Transcription 3 Activation


Abstract—Chronic angiotensin II (Ang II) infusion stimulates interleukin (IL) 6 release, and we and others have shown that preventing the increase in IL-6 significantly attenuates Ang II hypertension. This study measured renal blood flow (RBF) chronically, using Transonic flow probes in wild-type (WT) and IL-6 knockout (KO) mice, to determine the role of RBF regulation in that response. Ang II infusion at 200, 800, and 3600 ng/kg per minute caused a dose-dependent decrease in RBF in WT mice, and the response at 800 ng/kg per minute was compared between WT and IL-6 KO mice. Ang II infusion increased plasma IL-6 concentration in WT mice and increased mean arterial pressure (19 h/d with telemetry) from 113±4 to 149±4 mm Hg (Δ36 mm Hg) over the 7-day infusion period, and that effect was blocked in IL-6 KO mice (119±7 to 126±7 mm Hg). RBF decreased to an average of 61±8% of control over the 7-day period (control: 0.86±0.02 mL/min) in the WT mice; however, the average decrease to 72±6% of control (control: 0.88±0.02 mL/min) in the KO mice was not significantly different. There also was no difference in afferent arteriolar constriction by Ang II in blood-perfused juxtamedullary nephrons in WT versus KO mice. Phosphorylation of janus kinase 2 and signal transducer and activator of transcription 3 in renal cortex homogenates increased significantly in Ang II–infused WT mice, and that effect was prevented completely in Ang II–infused IL-6 KO mice. These data suggest that IL-6-dependent activation of the renal janus kinase 2/signal transducer and activator of transcription 3 pathway plays a role in Ang II hypertension but not by mediating the effect of Ang II to decrease total RBF. (Hypertension. 2010;56:879-884.)

Key Words: renal blood flow ■ angiotensin II ■ blood pressure ■ interleukin 6 ■ JAK2 ■ STAT3

Evidence linking inflammatory mechanisms to cardiovascular diseases, such as atherosclerosis1–4 and hypertension,5–9 continues to build. However, the identity of the precise inflammatory mediators and the mechanisms that underlie their cardiovascular actions remain unclear. The cytokine interleukin 6 (IL-6) is released from vascular tissue in response to angiotensin II (Ang II),10–13 and our laboratory showed that acute, stress-induced hypertension, which is Ang II dependent,14 is attenuated in IL-6 knockout (KO) mice.15 Therefore, we tested whether chronic Ang II hypertension depended on IL-6 and reported significant attenuation of Ang II hypertension in IL-6 KO mice.16 That was confirmed by Coles et al17 in IL-6 KO mice and wild-type (WT) mice with blockade of circulating IL-6. In addition, Guzik et al18 showed that generalized loss of T-cell–mediated inflammatory processes significantly attenuated Ang II hypertension. These data strongly implicated a role for IL-6 in Ang II hypertension. However, Guzik et al18 also showed that desoxycorticosterone acetate-salt hypertension was blunted by loss of T cells, and Luther et al19 reported that acute stimulation of IL-6 by Ang II infusion in human subjects was blocked by spironolactone. Those reports suggested the effect of IL-6 KO to attenuate Ang II hypertension could be because of an effect of IL-6 on mineralocorticoid-mediated actions. We tested that and reported that mineralocorticoid hypertension was not attenuated in IL-6 KO mice,20 thus supporting a specific link between IL-6 and Ang II hypertension.

The goal of this study was to determine whether an effect of IL-6 on Ang II–mediated renal vasoconstriction could be a potential mechanism for the dependence of Ang II hypertension on IL-6. IL-6 is required for Ang II–induced increases in vascular superoxide levels,21 and renal vascular actions in particular are supported by a report from Gadonski et al22 that IL-6 infusion increased plasma renin activity and decreased renal plasma flow and glomerular filtration rate in pregnant
rats. Therefore, we used chronically implanted renal artery flow probes to test the hypothesis that chronic Ang II–induced renal vasoconstriction would be attenuated in IL-6 KO mice. In addition, we measured the afferent arteriolar vasoconstrictor response to Ang II in anesthetized WT and IL-6 KO mice, using the blood-perfused juxtamedullary nephron preparation,23,24 and measured renal janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) phosphorylation.

Methods

Procedures involving animals were approved by the animal care and use committee of the Medical College of Georgia. The experiments were conducted in 12- to 14-week–old (23 to 28 g) male mice from Jackson Laboratories. The IL-6 KO mouse (B6.D2-SH2-Il6tm1Sfl/J) are on a C57BL/6 background, and those were the WT control mice that we used. Mice were allocated to 3 experiments.

Experiment 1: Blood Pressure

Mice were assigned randomly to 4 groups: Ang II infusion in IL-6 KO (n=6) and WT (n=8) mice and untreated control IL-6 KO (n=5) and WT (n=5) mice. Biotelemetry transmitter devices (Data Sciences, PA-C10) were implanted in the left carotid artery under isoflurane anesthesia using aseptic technique. Mice were transferred to a light- and temperature-controlled room in the animal facilities and were housed individually in standard mouse cages with tap water and standard rodent chow available ad libitum. They were given 5 to 7 days to recover from surgery before control measurements were made.

After 4 days of stable blood pressure, Ang II mice were implanted with micro-osmotic minipumps (ALZET 1007D) that delivered Ang II at 800 ng/kg per minute, and WT and KO mice were run simultaneously with minipumps filled with the same Ang II solution. On day 7 of infusion, mice were killed to collect kidney tissue and blood samples.

Experiment 2: Renal Blood Flow

There were 2 groups: Ang II infusion at 800 ng/kg per minute in IL-6 KO (n=6) and WT (n=5) mice. Under isoflurane anesthesia and using aseptic technique, a 0.5-mm Transonic flow probe (0.5 PSL) was implanted on the right renal artery via flank incision, with the mouse in a ventral recumbent position and with the probe cable running subcutaneously from the scapular region where the connector was anchored. To prevent the probe from twisting, we attached a small piece of brass shim stock (~3 x 6 mm²) to the underside of the probe using superglue before gas sterilization. This provided a stabilizing plate that was tucked under the kidney and surrounding fat, thus providing much more resistance to twisting. Recovery and housing were the same as described for experiment 1. Additional WT mice were run at 200 (n=3) and 3600 ng/kg per minute (n=8) to provide comparative information relative to the lowest Ang II dose reported by Kawada et al25 and Welch et al26 (200 ng/kg per minute). The high dose that we used in our previous study (3600 ng/kg per minute).16

Renal blood flow (RBF) was measured 4 to 6 hours each day by placing the mice individually in customized Plexiglas cages with a Dragonfly (model SL-88-10) electric swivel mounted on top. After 4 days of stable blood flow, all of the mice were implanted with micro-osmotic minipumps (ALZET 1007D) that delivered Ang II at 200, 800, or 3600 ng/kg per minute, and the WT and KO mice infused with 800 ng/kg per minute were run simultaneously using the same Ang II solution. After 7 days of infusion, the minipumps were removed for recovery period measurements. Recovery periods were used as the time control in this experiment to eliminate the need for running additional mice as control groups, and no tissues or plasma samples were taken from these mice.

Experiment 3: Afferent Arteriolar Diameter

Video microscopy experiments were conducted in vitro on IL-6 or WT mouse kidneys using the blood-perfused juxtamedullary nephron technique, as described previously.23,24 For each experiment, a mouse and rat were anesthetized with sodium pentobarbital (50 mg/kg IP). Perfusate blood was collected from a rat because it is not possible to obtain sufficient blood from blood donor mice for kidney perfusion. Afferent arteriolar diameters were measured at 12-second intervals. Sustained afferent arteriolar diameter was calculated from the average of measurements made during the final 2 minutes of each treatment period. After a 15-minute equilibration period, baseline afferent arteriolar diameters were measured. Each kidney was treated with increasing concentrations of Ang II (10⁻² to 10⁻³ mol/L), and diameter was monitored for 5 minutes per concentration.

Analytic Methods

Blood Pressure Measurement

Mouse cages were placed individually on Data Sciences receivers, and pulsatile arterial pressure was recorded from 3:00 PM to 10:00 AM (ie, 19 hours) each day. Analog signals from the transmitters were sampled for 5 seconds every 1 to 2 minutes at 500 Hz, and the average of those measurements was recorded as the daily mean arterial pressure (MAP) for each animal.

RBF Measurement

Mice were connected to a Transonic TS402 series flowmeter, and the signal was sampled continuously at 100 Hz using PowerLab and a Macintosh computer. The average of the entire collection period was used as that day’s RBF, and mice were excluded from the study if the flow signal was not pulsatile.

Plasma IL-6

Plasma IL-6 concentrations were measured by enzyme immunoassay (R&D Systems) from blood samples obtained by ventricular puncture under isoflurane anesthesia.

JAK2/STAT3: Tissue Homogenization for Protein Work

Tissues were quick frozen with liquid nitrogen, pulverized in a liquid nitrogen–cooled mortar and pestle, and solubilized in a composition of 255 mmol/L of sucrose per 10 mmol/L of Tris buffer (pH 7.4) with protease inhibitors (0.5 mmol/L of PMSF, 2 mmol/L of EGTA, 10 μg/μL of aprotonin, and 10 μg/mL of leupeptin) and tyrosine phosphatase (1 mmol/L of sodium orthovanadate) inhibitors. Homogenates were centrifuged (14 000 g for 10 minutes, 4°C), and supernatant total protein (Bio-Rad) was measured.

JAK2/STAT3: Western Blotting

Supernatants were separated on SDS-polyacrylamide gels (7.5% SDS-PAGE) and transferred to Immobilon-P membrane. Membranes were blocked and probed overnight (4°C) with primary antibody (1:1000, phosphospecific JAK2 and phosphospecific STAT3, Cell Signaling). Blots were washed, and an antirabbit horseradish peroxi-
dase–linked secondary antibody (1:7500, Amersham Laboratories) was added for 1 hour and incubated with the blots at 4°C. Blots were washed and enhanced chemiluminescence (Super Signals Ultra, Pierce) was used to visualize labeled bands. Blots were stripped and reprobed with the total antibody (1:1000, JAK2 BioSource, STAT3 BD Transduction Laboratories). β-Actin was used to ensure equal total protein loading between lanes. Band density was quantified using the program National Institutes of Health Image.

Statistical Analysis

Time- and treatment-dependent changes in MAP and RBF were analyzed with repeated-measures ANOVA. Significant F values were followed by the Dunnett test to determine specific within-group differences and completely randomized ANOVA plus Fisher protected least significant difference test on each day to determine specific between-group differences. JAK2/STAT3 data were analyzed with completely randomized ANOVA. Within-group analysis
of Ang II effects on afferent arteriolar diameter were made by 1-way ANOVA for repeated measurements combined with the Dunnett multiple range test. Across-group comparisons were made using the Student t test for unpaired data. Significance was P<0.05, and data are expressed as mean±SEM.

Results

Experiment 1: Blood Pressure

MAP averaged 113±4 mm Hg in WT mice during the control period and increased gradually during infusion of Ang II at 800 ng/kg per minute, plateauing after 3 to 4 days and averaging 149±4 mm Hg (Δ36 mm Hg) by day 7 (Figure 1). Control-period MAP in the KO mice averaged 119±7 mm Hg, and there was no significant change in MAP during Ang II infusion (average on day 7 was 126±7 mm Hg). There also was no change in MAP in the WT or KO control groups during the experiment (Figure 1).

Experiment 2: RBF

In the mice infused with Ang II at 800 ng/kg per minute, RBF averaged 0.86±0.02 and 0.88±0.02 mL/min in WT and KO, respectively, during the control period, and decreased significantly in both groups after starting Ang II infusion, averaging 61±8% and 72±6% of control over the 7-day period in WT and KO, respectively (Figure 2). However, despite that slightly lesser decrease in the KO mice and slight separation between KO and WT mice at days 1, 5, and 6, there was no statistically significant between-group difference between the WT and KO mice in the repeated-measures ANOVA for those 2 groups only or when their areas over the curve were compared by t test. When all 4 of the groups were tested together by ANOVA, there was a significant between-group F value but still no difference on any day between the WT and KO mice at 800 ng/kg per minute. Their areas over the curve together by ANOVA, there was a significant between-group F value but still no difference on any day between the WT and KO mice at 800 ng/kg per minute. Their areas over the curve were added to the assay to provide comparison with our previous reports,25,26 but RBF decreased significantly beginning on day 1 of Ang II in all of the other groups.

Experiment 3: Afferent Arteriolar Diameter

Ang II significantly decreased afferent arteriolar diameter in WT and IL-6 KO mice, beginning with the lowest concentration (0.1 nmol/L; Figure 3). There was a linear decrease in diameter with increasing Ang II concentration in both groups, reaching a nadir of ~80% of control diameter at an Ang II concentration of 10 nmol/L, and there were no differences between WT and KO mice at any concentration. Diameter returned to baseline in both groups after Ang II was removed.

Plasma IL-6 and JAK2/STAT3

Plasma IL-6 was below detection in all of the IL-6 KO mice and in the control WT mice and averaged 27.7±5.1 pg/mL in the WT mice infused with Ang II at 800 ng/kg per minute. Seven WT mice infused with Ang II at 3600 ng/kg per minute were added to the assay to provide comparison with our

Figure 1. MAP (mean±SEM) in WT and IL-6 KO mice infused with Ang II at 800 ng/kg per minute and in control WT and KO mice during the control (C) period and Ang II treatment (T) period. ∗P<0.05 vs baseline (within group).

Figure 2. RBF expressed as percentage of control (mean±SEM) in WT and IL-6 KO mice during the control (C) period, Ang II treatment (T) period, and recovery (R) period. WT mice were infused with Ang II at 200, 800, or 3600 ng/kg per minute, and the IL-6 KO mice were infused with Ang II at 800 ng/kg per minute. ∗P<0.05 vs baseline (within group); §P<0.05 vs 800 dose (between group); #P<0.05 vs 3600 dose (between group).

Figure 3. Afferent arteriole diameter in blood-perfused juxtaglomerular nephrons from WT and IL-6 KO mice in response to Ang II. *P<0.05 vs control (within group).
previous report, and indeed there was a significantly greater increase in plasma IL-6 concentration in those mice (48±9 pg/mL). Figure 4 shows significant activation (phosphorylation) of JAK2 and STAT3 in renal cortex of WT mice infused with Ang II at 800 ng/kg per minute and that effect was completely absent in the IL-6 KO mice.

**Discussion**

The main findings from this study are that chronic Ang II infusion in WT mice on normal salt intake caused dose-dependent stimulation of IL-6, significant hypertension, renal cortical JAK2/STAT3 phosphorylation, and dose-dependent decreases in RBF and afferent arteriole diameter. IL-6 KO completely prevented the hypertension and JAK2/STAT3 activation without causing significant attenuation of the renal vasoconstrictor response. These data show that Ang II–induced renal vasoconstriction does not require IL-6 and suggest that other actions likely contribute to the effect of IL-6 KO to prevent Ang II hypertension.

In the first study that showed attenuation of Ang II hypertension by KO of IL-6, we used a high Ang II infusion dose of 90 ng/min (=3600 ng/kg per minute) in mice on 4% salt intake. Therefore, one goal of this study was to measure RBF, and also blood pressure, under less severe conditions. However, it still was necessary to choose an Ang II dose that would decrease RBF over the time course (<1 week), where we knew IL-6 KO began to attenuate Ang II hypertension. Kawada et al. showed that Ang II infusion at 400 ng/kg per minute in mice did not cause hypertension or decrease RBF by day 6. Therefore, we used 800 ng/kg per minute, which is also similar to the dose used by Coles et al.

We found that Ang II infusion at that dose decreased RBF by ≈40% in WT mice over the 7-day infusion. That was surprising in itself, because even though Ang II is a well-known renal vasoconstrictor, we are not aware of any previous chronic RBF measurements in Ang II hypertension using a renal flow probe, and the magnitude and sustained nature of the decrease were remarkable. That result was one reason for adding WT mice at the Ang II doses of 200 and 3600 ng/kg per minute, and indeed the chronic renal vasoconstrictor effect of Ang II was dose dependent. However, the principal finding in this study is that IL-6 KO did not significantly attenuate the decrease in RBF at the dose of 800 ng/kg per minute. On the other hand, it is also apparent that the RBF plots in the KO versus WT mice are not superimposable, and that tends to focus attention on a comparison between those 2 RBF responses. The more remarkable observation, however, is that Ang II hypertension was prevented completely in the IL-6 KO mice, yet they still had a significant decrease in RBF.

This lack of effect of IL-6 KO on Ang II–induced renal vasoconstriction was surprising in light of the reported effect of IL-6 infusion to increase blood pressure and cause renal vasoconstriction in pregnant rats. In addition, IL-6 is linked to pulmonary hypertension, it is required for Ang II–mediated increases in vascular superoxide, and we showed that acute-stress hypertension in mice, which is Ang II dependent, is blunted in IL-6 KO mice. Crosswhite and Sun also have just reported that knockdown of IL-6 decreased blood pressure and vascular superoxide production in rats with cold-induced hypertension, which is an Ang II–dependent model. Moreover, for IL-6 KO not to attenuate the decrease in RBF, yet completely block the hypertension, means that some decrease in tubular sodium reabsorption must have occurred during Ang II infusion in those mice, and we do not have an explanation for that. Yet, despite these arguments, and even if one speculates that additional mice in the IL-6 KO group would have yielded a statistically significant difference between the WT and KO mice, there still would remain a significant decrease in RBF caused by Ang II infusion in the IL-6 KO mice. The use of chronically implanted flow probes, the recovery data, and the lack of effect of Ang II infusion at 200 ng/kg per minute assuage concerns about the validity of that finding.

However, if the blood pressure and blood flow data from experiments 1 and 2 are used to estimate renal vascular resistance during Ang II infusion, it would be lower in the IL-6 KO mice (lower MAP with no difference in RBF).
Therefore, although Ang II significantly decreased RBF in the IL-6 KO mice, the lesser increase in renal vascular resistance could indicate impaired vasoconstrictor responses to Ang II in IL-6 KO mice. The data from Inscho’s laboratory (Figure 3) argue against that, by showing no difference in the afferent arteriolar constrictor effect of Ang II in WT versus IL-6 KO mice. In addition, Coles et al.15 showed that there was no difference in the contractile response to Ang II in aortic rings isolated from WT versus IL-6 KO mice. These data are consistent with our data that showed no significant difference in the Ang II–induced decrease in RBF in WT versus IL-6 KO mice. This suggests that other factors, such as autoregulation, may have contributed to greater estimated renal vascular resistance in the hypertensive WT mice.

These results do not completely rule out potential vascular actions of IL-6, such as mediating effects of Ang II on filtration fraction or medullary blood flow. The fact that the RBF responses in WT versus KO mice at 800 ng/kg per minute were not superimposable suggests that there even may have been mild attenuation of more global Ang II–induced vasoconstriction that simply was not statistically significant at this infusion dose. In addition, comparing the RBF and MAP responses to Ang II in this study and previous studies16,17,25,26 suggests that there could be different thresholds for IL-6 action on Ang II hypertension versus renal vasoconstriction. Therefore, it is possible that there is a level of Ang II hypertension that is associated with, and perhaps dependent on, IL-6–mediated vasoconstriction.

The effect of IL-6 KO to prevent Ang II hypertension without preventing the decrease in RBF nonetheless is strong evidence that renal vasoconstriction likely is not the primary explanation for the blood pressure effect. However, the inhibition of renal JAK2/STAT3 phosphorylation by IL-6 KO still implicates a role for the kidneys. JAK2/STAT3 signaling has been shown to be an early upstream component of the renal and glomerular actions of Ang II in diabetes mellitus.31–33 and Guilluy et al.34 reported recently that Ang II hypertension was attenuated by chronic blockade of JAK2 activation. Satou et al.35 showed that Ang II alone did not cause STAT3 phosphorylation in a cultured human proximal tubular cell line but did potentiate the stimulatory action of IL-6. This suggests that IL-6 could play a role in the effect of Ang II on tubular sodium reabsorption, and it is consistent with evidence that IL-6 activates the collecting duct epithelial sodium channel16 and increases sodium-glucose cotransporter expression and STAT3 phosphorylation in primary cultured renal proximal tubular cells.37 There is no direct evidence that IL-6 contributes to Ang II–mediated regulation of tubular reabsorption, but it is intriguing to consider based on those studies, our in vivo evidence that renal JAK2 and STAT3 are activated in Ang II hypertension and require IL-6, and our evidence that IL-6 KO can attenuate Ang II hypertension without blocking renal vasoconstriction.

**Perspectives**

The effect of T-cell elimination to attenuate Ang II and mineralocorticoid hypertension, together with the effect of IL-6 elimination to attenuate only Ang II hypertension, suggests that branching down from more global levels of the inflammatory response reveals more specific relationships between inflammatory mediators and certain types of hypertension. Likewise, our findings that IL-6 KO can completely block Ang II–induced JAK2/STAT3 activation and hypertension without blocking Ang II–induced decreases in RBF suggest that inflammatory mediators, such as IL-6, may have dose- and tissue-specific mechanisms for controlling the response to hypertensive stimuli. That also is supported by the effect of IL-6 KO to cause 50% inhibition of Ang II hypertension at the 3600-ng/kg-per-minute dose16 but completely block it at the 800-ng/kg-per-minute dose. It will be important now to determine whether the in vitro evidence supporting renal tubular actions of IL-6 translate to effects on Ang II–induced increases renal sodium reabsorption in vivo, and to determine whether such effects are amplified at lower Ang II infusion doses.

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**Disclosures**

None.

**References**

12. Han Y, Runge MS, Brasier AR. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic acti-
The role of angiotensin II on vascular tone and blood pressure.


Angiotensin II hypertension is attenuated in interleukin-6 knockout mice. Hypertension. 2004;44:259–263.


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